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mtDNA diversity in a rabbit population from Sicily (Italy)

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Abstract: The European rabbit *Oryctolagus cuniculus* (*O. c.*) is a small game species found in several parts of the world and represents an important resource for many predators. It has been classified as a Near-Threatened species on the Red List of Italian Vertebrates even though it is also considered to be an agricultural pest species in many areas. It is a polytypic species present as two known subspecies: *O. c. cuniculus* and *O. c. algirus*. The Italian geographical distribution of the two subspecies is known, but mostly based on morphological, biogeographic, and historical literature data. In Sicily, there is no complete genetic description of the actual existing subspecies; previous studies have only reported the differences in size of the Sicilian rabbit population. In this study, we analyzed genetic data within a phylogenetic framework through mitochondrial (mt) cytochrome *b* (cyt *b*) DNA from 13 rabbit samples collected from different sites in Sicily. We reconstructed the intraspecific phylogeny by comparing cyt *b* mtDNA sequences of 13 newly isolated *O. cuniculus* haplotypes from Sicily and 7 individuals from other areas (Canada, France, Mexico, North Italy, South Africa, Spain, Sweden). Our results show that the rabbit population from Sicily has a mitochondrial type (lineage B) previously shown to be associated with *O. c. cuniculus*, which is similar to sequences from rabbits in Northeast Spain, South France, Sweden, and South Africa.

Key words: Biodiversity, rabbit taxonomy, cyt *b* mitochondrial DNA, *Oryctolagus cuniculus*

1. Introduction

The European rabbit *Oryctolagus cuniculus* (*O. c.*) is traditionally one of the most important small game species in the Mediterranean Basin. It is also considered a multifunctional keystone species for natural and cultivated Mediterranean ecosystems (Delibes-Mateos et al., 2008). It is present in Europe, North Africa, South America, Australia, and New Zealand (Ferrand, 2008). Currently, the species has been classified and given the status of Near-Threatened at the international level (http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T41291A10415170.en).

In Italy, the European rabbit has been classified as a Near-Threatened species on the Red List of Italian Vertebrates (IUCN, 2014; available at www.iucnredlist.org).

The European rabbit was quite common in central and southern Europe during the late Upper Pleistocene. At the end of the last glaciation the geographical distribution of this species was limited to the Iberian Peninsula (IP) and probably the south of France (SF) (Kaetzke et al., 2003; Lopez-Martinez, 2008), where there were likely two glacial refugia; thus, as a consequence, two divergent genetic lineages evolved. The signatures of this are evident throughout the genome and can be observed in the analysis of allozyme variation, mtDNA variation, the Y-chromosome, the X-chromosome, and autosomes (Branco et al., 2000; Geraldes et al., 2006, 2008; Ferrand and Branco, 2007; Carneiro et al., 2010, 2014a, 2014b). The European rabbit is recognized formally in two subspecies, *O. c. cuniculus* (L., 1758) and *O. c. algirus* (Loche, 1858), as described by Ferrand (2008).

The species later spread throughout almost all of the world as a result of human activity (Flux, 1994). Specifically, during the 2nd or 3rd centuries AD, the European rabbit was introduced onto the central Mediterranean islands, including Sicily (Flux and Fullagar, 1992; Flux, 1994; Kaetzke et al., 2003), probably by the Romans (Bodson,

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1978). After its introduction, the only evidence of the presence of the European rabbit in Sicily is represented by fossils found at the Brucato site during the Middle Ages (13th–14th centuries) (Barrett-Hamilton, 1912; Constable, 2003). At this site, archaeological research has revealed that about 40% of the animals hunted by humans consisted of rabbits (Bresc, 1980). Thanks to the income of "Venatio cuniculorum", of which there are different statements, it is known that rabbits abounded in many other places in Sicily. In the Mediterranean Basin, there is indication of the presence of the European rabbit from archaeological exploration on the islands of Nisida and Capri (Naples, Italy), and Zembra (Tunisia) (Flux and Fullagar, 1992; Flux, 1994; Kaetzke et al., 2003).

Today, in the Sicilian region of Italy, as well as in other European countries (Millán et al., 2012), the European rabbit is at the center of serious management and conservation problems. In fact, this lagomorph is very important for the conservation of the endangered Sicilian population of raptors such as Bonelli's eagle (*Hieraaetus fasciatus*), and also for very generalist species such as the common buzzard (*Buteo buteo*) and red fox (*Vulpes vulpes*) (Caruso and Siracusa, 2001; Moleón et al., 2012; Lopez-Lopez et al., 2012).

According to the Guidelines for the Release of Wildlife Species (INFS, 2007), the rabbit is considered to be a para-autochthonous species, as it was introduced before 1500 and is therefore now contextualized in the local fauna landscape (Lees and Bell, 2008). Nevertheless, in some areas of Sicily and on the nearest small islands, where there are no predators and in a nature reserve where hunting is prohibited, the European rabbit is a pest species inducing damage to the agroecosystem and a decrease in the plant population, and causing considerable irreversible ecological and economic damage (Lees and Bell, 2008; Cooke, 2012).

In Sicily, as well as in the rest of Europe, the reduction in the rabbit population was probably due to myxomatosis and rabbit haemorrhagic disease (reviewed by Abrantes et al., 2012) recorded in 1986. In this context, and mainly for hunting purposes, regional authorities have released about 10,000–15,000 captive-raised rabbits of uncertain origin every year. The population of European rabbit living in Sicily and on the nearest small islands seems to belong to the subspecies *O. c. huxleyi* (Haeckel 1874), which is synonymous for *O. c. algirus*, according to Toschi (1965). However, this statement is based only on the biometric analysis of the body size. Recently, Lo Valvo et al. (2014) examined 7 body variables and 23 cranial variables of 166 and 120 individuals, respectively, and compared the results with biometric data from other European populations. They showed that the European rabbit currently present in Sicily does not have a small body size, as assumed by Toschi (1965), but rather has a medium body size as in current populations living in northern Spain and southern France, which are referred to as *O. c. cuniculus* (Callou, 2002; Ferreira et al., 2015).

It is clear that the European rabbit population in Sicily has never been carefully managed, mainly because of very poor knowledge of its biology and ecoethology in the wild, and also because of a strong uncertainty about its genetic, morphometric, and biogeographic characteristics. To start filling these gaps, we worked on a research project on the European rabbit in Sicily, studying the genetics of the Sicilian *O. c.* by means of a marker gene, namely the mitochondrial DNA (mtDNA) of the cytochrome *b* (cyt *b*) gene, which is commonly used in phylogenetics to determine evolutionary relationships between organisms due to its DNA sequence divergence. It is considered one of the most useful genes in determining relationships within families and genera as demonstrated for mammals (Castresana, 2001; Tobe et al., 2010), and within subspecies, such as *Lepus corsicanus* (Pierpaoli et al., 1999). In the mitochondrion, the cyt *b* gene encodes for an integral membrane protein of approximately 400 amino acid residues; it is a component of the respiratory chain complex III, also known as the bc1 complex, involved in the electron transport of protons (Howell, 1989; Degli Esposti et al., 1993). Since it is possible to perform genetic studies about species evolutionary history, we used the mitochondrial cyt *b* gene known as a valid marker of intra- and interspecific variation (Degli Esposti et al., 1993; Tobe et al., 2010).

mtDNA is a maternally inherited nonrecombinant molecule and therefore, in species that hybridize, only provides information on the ancestry of the female lineage. As an example, it was used to establish introgression cases for hares in the IP; in fact, a high frequency of *Lepus timidus* mtDNA is observed in individuals from native hares, but *Lepus timidus* itself no longer occurs in the IP (Melo-Ferreira et al., 2009).

In this study, we applied genetic analysis as a complement to the phenotypic descriptors of the *O. c.* Sicilian population investigated before. We present the results on Sicilian rabbit phylogeny by comparing cyt *b* mtDNA and protein sequences with homologs from European populations with the aim to better understand their taxonomic relationships and to specify which lineage (A or B) the Sicilian rabbit belongs to. The island of Sicily is at the border of the distribution of *O. c. cuniculus* in the area of the Italian Peninsula. Therefore, depending on the subspecies of the Sicilian population, the geographic ranges would be confirmed or modified.

2. Materials and methods

2.1. Tissue sample collection

Between 1997 and 2006, European rabbit tissue specimens obtained from hunters were collected from different sites in the Sicilian provinces, including some small islands (such as Ustica in the province of Palermo and Vulcano in the province of Messina). The specimens were collected as representative samples from about 50 rabbits from diverse sites in different geographically distributed Sicilian provinces. Twenty samples were then amplified and sequenced, 13 of which were found to have different DNA sequences; for this reason, they were selected for the purpose of this study. The cyt *b* mtDNA partial sequence, 954 bp in length (out of the whole coding region of 1140 bp) was analyzed. In Table 1 and Figure 1A, the Sicilian sites are described in detail, together with the relative provinces of sampling, the geographic coordinates (UTM WGS84), and the size of the isolated DNA fragments. Two samples are smaller in size, probably due to a polymerase failure, being 921 and 927 nt long, from Isola delle Femmine (Palermo) and Oasi Scala (Caltanissetta), respectively.

Animals were treated in accordance with the guidelines of the local ethics committee.

2.2. mtDNA extraction and polymerase chain reaction (PCR) of the *cyt b* gene

The mtDNA was extracted from ear and muscle samples using the genomic DNA Purification Kit (Fermentas) according to the manufacturer's guidelines. An aliquot of DNA was amplified by using conventional PCR approaches. PCR was performed with Taq (Invitrogen) polymerase in 25-µL reactions under the following conditions: 1 cycle at 95 °C for 5 min; 30 cycles at 95 °C for 45 s (denaturation), 55 °C for 30 s (annealing), and 72 °C for 60 s (extension); and 7 min at 72 °C (final extension). Specific oligonucleotides used to amplify mtDNA were designed as follows: forward 5'-ATCCAACATCTCTGCTTGATG-3' and reverse 5'-GGTTGGCCTCCGATTCATGT -3'. The PCR reaction products were DNA fragments ranging from 921 to 954 nt that were purified by Exosap (BMR Genomics Service, Padua, Italy) and sequenced in both directions by MWG–Operon Sequencing Service (Germany). Sequences were deposited in GenBank and the comparison of sequences

Table 1. *O. cuniculus* individual names, A/B lineage assignment, geographical sites and coordinates, mtDNA length, and relative GenBank accession number.

Name	A/B ineage	Site	Sicilian province	UTMX	UTMY	DNA size (nt)	Accession number
1-Sicily	B	Cianciana	Agrigento	362000	4151400	954	HG810781.1
2-Sicily	B	OasiScala	Agrigento	395000	4156700	927	HG810791.1
3-Sicily	B	Realmonte	Agrigento	364300	4129500	954	HG810788.1
4-Sicily	B	Butera	Caltanissetta	428400	4120000	954	HG810780.1
5-Sicily	B	Terrasini	Palermo	330300	4223300	954	HG810785.1
6-Sicily	B	Giacalone	Palermo	345100	4211600	954	HG810782.1
7-Sicily	B	Nociazzi	Palermo	415800	4185100	954	HG810784.1
8-Sicily	B	Pizzo Inserra	Palermo	350500	4225000	954	HG810790.1
9-Sicily	B	Marsala	Trapani	280500	4185000	954	HG810783.1
10-Sicily	B	Segesta	Trapani	309300	4203100	954	HG810787.1
11-Sicily	B	Femmine Is.	Palermo	345600	4230600	921	HG810779.1
12-Sicily	B	Ustica Is.	Palermo	341100	4284600	954	HG810786.1
13-Sicily	B	Vulcano Is.	Messina	498500	4250000	954	HG810789.1
Lineage A (*O. c. algirus*)	A		Spain, south			954	AJ243096.1
Lineage B (*O. c. cuniculus*)	B		France			954	AJ243197.1
Italy	B		Italy			946	AF157467.1
Mexico	A		Mexico			954	HQ596486.1
Canada	B		Canada			954	U07566.1
Sweden	B		Sweden			954	AJ001588.1
South Africa	B		South Africa			954	AY292717.1
Lepus europaeus			Sweden			954	AJ421471.1
Lepus corsicanus			Italy			949	AF157463

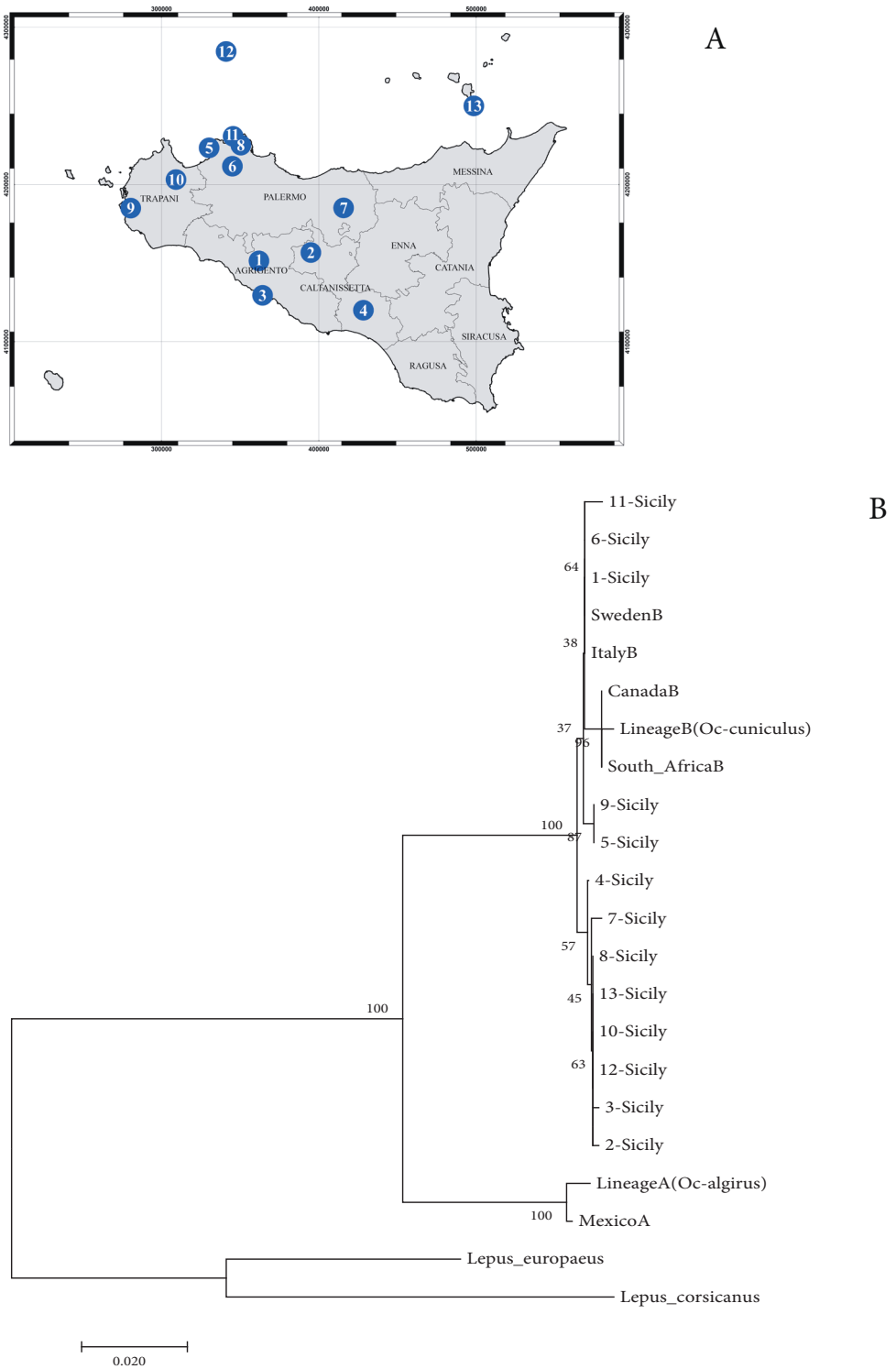

Figure 1. A) Map of sampling sites in Sicilian provinces. Numbers correspond to sampling sites described in Table 1. B) Evolutionary relationships of taxa inferred by MEGA 5 program: neighbor-joining phylogenetic tree, derived from cyt *b* mtDNA multiple alignment shown in Figure S1, of *O. cuniculus* samples derived from the sites listed in Table 1. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. Bar = 0.02. Oc-cuniculus = *O. cuniculus cuniculus*; Oc-algirus = *O. cuniculus algirus.*

was performed using the Blast program (Altschul et al., 1990). Restriction analysis was performed with the NEB Cutter 2.0 program (Vincze et al., 2003).

2.3. Phylogenetic analysis

DNA and protein alignments were performed using Clustal W2 software (Larkin et al., 2007). Shading of the multiple alignment of the selected sequences was obtained by using the Boxshade 3.21 program (http://www.ch.embnet.org/software/BOX_form.html). On the basis of the alignment in supplementary Figure S1, we constructed a phylogenetic tree. *L. corsicanus* and *L. europaeus* were used as outgroups to root the tree. The evolutionary profile was determined using the MEGA 5 program (Tamura et al., 2011) and the neighbor-joining method (Saitou and Nei, 1987). The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The program DnaSP (Version 5.0) (Librado and Rozas, 2009) was used to sort haplotypes and analyze the number of variable sites (S), number of haplotypes (h), and haplotype diversity (Hd).

3. Results

The genetics of the Sicilian population of *O. c.* was studied using the cyt *b* mtDNA sequence, as described in Section 2. First, we compared the 13 isolated sequences with those of GenBank by using the Blast program, and we found that all the Sicilian sequences showed a high similarity (equal to 99%) to *O. c. cuniculus* (L., 1758), called lineage B by Branco et al. (2000). From our results, the reciprocal comparison between the cyt *b* nucleotidic sequences from the two annotated lineages, A (corresponding to *O. c. algirus*) and B (corresponding to *O. c. cuniculus*), showed 93% homology. Identity decreased to 85% when both sequences were compared with the *Lepus* species. The nucleotidic comparison between cyt *b* sequences from the Sicilian population and from other populations found in GenBank showed a high sequence identity (equal to 99%) with the sequences from France and South Africa, Canada, Sweden, and North Italy, and a lower sequence identity (equal to 93%) with sequences from Andalusia (South Spain) and Mexico.

We then compared the newly sequenced clones with a selected series of sequences found in GenBank from the two subspecies, *O. c. cuniculus* (L., 1758) and *O. c. algirus* (Loche, 1858), that for Branco et al. (2000) correspond to lineage A. In particular, the cyt *b* genes pertaining to both A and B lineages from other areas of the world such as Sweden, France, southern Spain, Mexico, southern Africa, Canada, and northern Italy were considered. The ClustalW2 multiple alignment of all 22 sequences analyzed here is shown in supplementary Figure S1. The results of the shading program in supplementary Figure S2 (where the different nucleotides of Sicilian sequences with respect to lineage A are shaded) showed that there is intraspecific divergence for the mitochondrial cyt *b* gene. This divergence was higher between all the Sicilian nucleotidic sequences and lineage A with respect to lineage B.

The graphic phylogram (Figure 1B) shows that the main branch divides into two clades: one for lineage A and one for lineage B. Lineage B divides further into two branches: one giving rise to a group of 8 Sicilian sequences and another containing 5 Sicilian sequences besides those from France, South Africa, Canada, Sweden, and northern Italy. The higher bootstrap values indicate the accuracy of the data.

Polymorphic and haplotype analyses are shown in Table 2. A total of 75 variable sites were detected out of 954 total nucleotides. A total of 10 different haplotypes out of 15 analyzed sequences was sorted, indicating that individuals 10-Sicily/12-Sicily/13-Sicily/8-Sicily, 1-Sicily/6-Sicily, and 5-Sicily/9-Sicily were identical respectively, considering the mtDNA sequence analyzed here.

A genetic analysis of mitochondrial cyt *b* nucleotidic sequences conducted by analyzing the single cutter restriction enzyme analysis supported our investigations. Figure S1 highlights, in different colors, some of the most interesting and indicative single cutter restriction enzyme sites and their positions. Among the differences, a very interesting finding was the discovery that all Sicilian sequences contained an EcoRI restriction site at the same position (nucleotide 558 of the partial sequence considered here), a feature that was shared with lineage B, northern Italy, Canada, South Africa, and Sweden, but was in contrast to the sequences of lineage A and Mexico, which had no EcoRI site, as well as *L. europaeus*. The Sicilian sequences shared other typical restriction sites (HincII 148, AluI 806, DrdI 888) in an identical position with lineage B and were dissimilar to lineage A; the latter does not possess the DrdI site, and it possesses the other restriction sites in different positions (specifically, AluI 500 and HincII 439). The same Sicilian individuals did not contain a SalI restriction site, which is present only in Mexico and lineage A (at the same position of 437 of the partial sequence considered here) (see Figure S1).

4. Discussion

In this study, we applied genetic analysis as a complement to the previously defined phenotypic descriptors of the *O. c.* Sicilian population (Lo Valvo et al., 2014), by using the high variable region of cyt *b* mtDNA. In fact it rapidly accumulates nucleotidic substitutions, producing variants of mtDNA present in a single species, called haplotypes (Ingman et al., 2000). Cyt *b* mtDNA has been frequently used in mammals (Barome et al., 1998), including the wild rabbit from the IP (Branco et al. 2000), whereas this is the

Table 2. Genetic diversity estimates for *Oryctolagus cuniculus* from Sicily mtDNA.

Number of sequences	15
Number of sites	954
Number of polymorphic (segregating) sites	S: 75
Site positions	4 17 18 37 46 65 73 148 151 154 163 169 208 211 244 262 274 280 298 313 316 319 325 337 349 352 376 430 439 442 454 463 482 484 526 541 553 562 565 575 589 592 601 611 617 622 629 635 638 652 673 677 697 706 727 735 742 761 763 808 814 815 820 850 856 892 908 910
Number of haplotypes	h: 10
Haplotype diversity	Hd: 0.924
Hap_1: 4	[10-Sicily 12-Sicily 13-Sicily 8-Sicily]
Hap_2: 1	[11-Sicily]
Hap_3: 2	[1-Sicily 6-Sicily]
Hap_4: 1	[2-Sicily]
Hap_5: 1	[3-Sicily]
Hap_6: 1	[4-Sicily]
Hap_7: 2	[5-Sicily 9-Sicily]
Hap_8: 1	[7-Sicily]
Hap_9: 1	[Lineage A *O. c. algirus*]
Hap_10: 1	[Lineage B *O. c. cuniculus*]
Standard deviation of haplotype diversity	0,053

first time it has been used for studying the taxonomy of the Sicilian *O. c.* population.

In addition to morphological data actually used to understand the phylogenetic characteristics of lagomorphs (Lo Valvo et al., 2014; Ge et al., 2015), the genetic and phylogenetic analyses have enabled variations in the genotypic traits to be identified. Here, genetics was useful to analyze the Sicilian *O. cuniculus* population and overall to distinguish which lineage this population belongs to out of the existing ones: lineage A (*O. c. algirus*) and lineage B (*O. c. cuniculus*), as described by Branco et al. (2000). Due to the possible intake of genetically different rabbits, which could cause a hybrid population with more health problems than the native Sicilian population that has evolved and already adapted itself to the environment, this new information can help to plan future strategies for the correct management and conservation of the Sicilian rabbit.

This study can also serve to stimulate further investigations of the genetic history of the rabbit in Sicily in order to reconstruct rabbit phylogeography in the Mediterranean, which has yet to be fully understood.

We conclude that the results related to the mtDNA analysis are consistent with the hypothesis that the Sicilian rabbit is more similar to *O. c. cuniculus*, in agreement with morphological data (Lo Valvo et al., 2014), and in disagreement with Toschi (1965), who ascribed the Sicilian rabbit population to the *O. c. algirus* subspecies. Accordingly, Hardy et al. (1994) reported on rabbits from Zembra (Tunisia), a Mediterranean island near Sicily, belonging to the *O. c. cuniculus* subspecies. In a more recent study, rabbits from the island of Mallorca were identified as belonging to *O. c. cuniculus*, having origins from Iberian and French populations (Seixas et al., 2014). Instead, the IP population in the northeast was regarded as *O. c. cuniculus*, whereas populations in the southwest of the IP, North Africa, the Mediterranean islands (including Sicily), and the Portuguese Atlantic islands were considered as *O. c. algirus* (Branco et al., 2000; Esteves et al., 2004). All other introduced populations and domesticated varieties were considered to be descendants of the nominate subspecies (Monnerot et al., 1994; Branco et al., 2000; Branco and Ferrand, 2003).

Nevertheless, it is conceivable that an original form of *O. c. algirus* in Sicily has undergone a simple hybridization or a gene introgression after the introduction of *O. c. cuniculus* even if we have never found hybrids in Sicily. There has, moreover, not been sufficient time to determine whether *O. c. cuniculus* has been substituted by *O. c. algirus* because of the very recent intake of new rabbits on the island (in the 1980s). Thus, we hypothesize that the only species that has always lived in Sicily could be *O. c. cuniculus*.

Since mitochondrial DNA is inherited through the maternal line, given the large number of rabbits raised on Sicilian territory and the complex dynamics due to human-mediated introductions, only careful analysis conducted on genomic DNA can show whether the population of wild Sicilian rabbits belongs to a nominate subspecies or whether it is a hybrid population. If the second hypothesis were true, then this population would be a remarkable case study for evolutionary and conservation biology.

Furthermore, the Mediterranean-type ecosystem is characterized by hot, dry summers and mild, rainy winters. It is considered a very important center of biodiversity, after the tropics, and comprises the Italian Mediterranean Sea and the IP (Cowling et al., 1996), known as places of refuge for temperate species during past glaciations, as well as for rabbits (Taberlet et al., 1998).

Consequently, examination of the ecological and economic importance of this lagomorph in Sicily, and the study of its genetic diversity compared to its geographical distribution, can help in designing adequate conservation and management strategies and in preserving ecosystems and biodiversity.

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CLUSTAL 2.1 multiple sequence alignment


LineA_Oc-algirus_       ATCCAACATCTCTGCTTGATGAAACTTTGGCTCTCTGCTAGGCCTATGCCTTATAATTCA 60
MexicoA                 ATCAAACATCTCTGCCTGATGAAACTTTGGCTCTCTGCTAGGCCTATGCCTTATAATTCA 60
12-Sicily               ATCAAACATCTCTGCCTGATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 60
2-Sicily                ATCAAACATCTCTGCCTGATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 60
10-Sicily               ATCAAACATCTCTGCCTGATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 60
13-Sicily               ATCAAACATCTCTGCCTGATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 60
8-Sicily                ATCAAACATCTCTGCCTGATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 60
3-Sicily                ATCAAACATCTCTGCCTGATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 60
7-Sicily                ATCAAACATCTCCACCTGATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 60
4-Sicily                ATCAAACATCTCTGCCTGATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 60
LineB_Oc-cuniculus_     ATCAAACATCTCTGCCATATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 60
CanadaB                 ATCAAACATCTCTGCCTGATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 60
South.AfricaB           ATCAAACATCTCTGCCTGATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 60
ItalyB                  --------TCTCTGCCTGATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 52
SwedenB                 ATCAAACATCTCTGCCTGATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 60
1-Sicily                ATCAAACATCTCTGCCTGATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 60
6-Sicily                ATCAAACATCTCTGCCTGATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 60
9-Sicily                ATCAAACATCTCCACCTGATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 60
5-Sicily                ATCAAACATCTCCACCTGATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 60
11-Sicily               ATCAAACATCTCTGCTTGATGAAACTTTGGCTCTCTACTAGGCCTGTGCCTTATAATTCA 60
Lepus.europaeus         ATCAAACATCTCTGCCTGATGAAACTTCGGCTCTCTATTGGGATTATGCCTAATAATCCA 60
Lepus.corsicanus        -----ACATTTCAGCCTGATGAAACTTTGGCTCCCTATTAGGACTATGCCTAATAATCCA 55
                              * **  *   ********* ***** **  * **  * ***** ***** **

LineA_Oc-algirus_       AATTCTCACTGGTCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
MexicoA                 AATTCTCACTGGTCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
12-Sicily               AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
2-Sicily                AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
10-Sicily               AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
13-Sicily               AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
8-Sicily                AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
3-Sicily                AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
7-Sicily                AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
4-Sicily                AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
LineB_Oc-cuniculus_     AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
CanadaB                 AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
South.AfricaB           AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
ItalyB                  AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 112
SwedenB                 AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
1-Sicily                AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
6-Sicily                AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
9-Sicily                AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
5-Sicily                AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
11-Sicily               AATTTTCACTGGCCTATTCTTAGCCATACACTACACCTCTGACACAACAACAGCATTCTC 120
Lepus.europaeus         GATCCTAACTGGCTTATTTCTAGCCATACACTACACATCAGACACAGCTACAGCATTCTC 120
Lepus.corsicanus        AATCCTAACTGGCCTGTTCCTAGCTATACACTACACATCAGATACAGCAACAGCATTTTC 115
                         **  * *****  * **  **** *********** ** ** *** * ******** **
                                                HincII
LineA_Oc-algirus_       ATCAGTAACCCATATTTGCCGAGATGTAAATTACGGCTGACTCATCCGGTACCTCCACGC 180
MexicoA                 ATCAGTAACCCATATTTGCCGAGATGTAAATTACGGCTGACTCATCCGGTACCTCCACGC 180
12-Sicily               ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 180
2-Sicily                ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 180
10-Sicily               ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 180
13-Sicily               ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 180
8-Sicily                ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 180
3-Sicily                ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 180
7-Sicily                ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 180
4-Sicily                ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 180
LineB_Oc-cuniculus_     ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 180
CanadaB                 ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 180
South.AfricaB           ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 180
ItalyB                  ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 172
SwedenB                 ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 180
1-Sicily                ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 180
6-Sicily                ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 180
9-Sicily                ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 180
5-Sicily                ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 180
11-Sicily               ATCAGTAACCCATATTTGCCGAGATGTTAACTATGGCTGACTTATCCGATACCTCCACGC 180
Lepus.europaeus         CTCAGTTACACATATTTGCCGAGATGTAAACTACGGCTGACTCATTCGTTACTTACACGC 180
Lepus.corsicanus        TTCAGTCACACATATTTGCCGAGACGTAAACCATGGCTGACTTATTCGTTACCTACACGC 175
                         ***** ** ************** ** **  * ******** ** ** *** * *****

LineA_Oc-algirus_       TAACGGAGCATCTATATTCTTTATTTGTCTTTACATACACGTAGGCCGCGGAATCTACTA 240
MexicoA                 TAACGGAGCATCTATATTCTTTATTTGTCTTTACATACACGTAGGCCGCGGAATCTACTA 240
12-Sicily               TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 240
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Figure S1. Multiple sequence alignment of *Oryctolagus cuniculus* cyt *b* mtDNA from different individuals. GenBank accession numbers are indicated in Table 1. The single enzyme restriction sites are highlighted in colors/gray.

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2-Sicily                TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 240
10-Sicily               TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 240
13-Sicily               TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 240
8-Sicily                TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 240
3-Sicily                TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 240
7-Sicily                TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 240
4-Sicily                TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 240
LineB_Oc-cuniculus_     TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 240
CanadaB                 TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 240
South.AfricaB           TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 240
ItalyB                  TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 232
SwedenB                 TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 240
1-Sicily                TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 240
6-Sicily                TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 240
9-Sicily                TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 240
5-Sicily                TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 240
11-Sicily               TAACGGAGCATCTATATTCTTTATTTGCCTCTACATACACGTAGGCCGCGGAATCTACTA 240
Lepus.europaeus         TAATGGAGCATCAATATTCTTTATTTGCTTATATATACATGTAGGCCGTGGAATCTACTA 240
Lepus.corsicanus        CAACGGAGCATCAATATTTTTTATCTGCTTATATATACATGTAGGTCGTGGAATCTACTA 235
                         ** ******** ***** ***** **  * ** ***** ***** ** ***********

LineA_Oc-algirus_       TGGGTCATACACATACCTAGAAACCTGAAACATCGGCATTATCCTCCTATTCGCAGTGAT 300
MexicoA                 TGGGTCATACACATACCTAGAAACCTGAAACATCGGCATTATCCTCCTATTCGCAGTGAT 300
12-Sicily               TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 300
2-Sicily                TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 300
10-Sicily               TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 300
13-Sicily               TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 300
8-Sicily                TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 300
3-Sicily                TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 300
7-Sicily                TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 300
4-Sicily                TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 300
LineB_Oc-cuniculus_     TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 300
CanadaB                 TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 300
South.AfricaB           TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 300
ItalyB                  TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 292
SwedenB                 TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 300
1-Sicily                TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 300
6-Sicily                TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 300
9-Sicily                TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 300
5-Sicily                TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 300
11-Sicily               TGGATCATACACATACCTAGAGACCTGAAACATTGGCATCATCCTCCTATTCGCAGTAAT 300
Lepus.europaeus         CGGCTCATATACTTACCTAGAAACCTGAAACATTGGCATTATTCTACTATTCGCAGTAAT 300
Lepus.corsicanus        TGGCTCATATACTTACCTAGAAACCTGGAATATTGGCATTATTCTTCTATTTGCAGTAAT 295
                         ** ***** ** ******** ***** ** ** ***** ** ** ***** ***** **

LineA_Oc-algirus_       AGCCACAGCATTCANGGGATATGTTCTCCCATGAGGTCAAATATCATTTTGAGGAGCAAC 360
MexicoA                 AGCCACAGCATTCATGGGATATGTTCTCCCATGAGGTCAAATATCATTTTGAGGAGCAAC 360
12-Sicily               AGCCACAGCATTTATGGGTTATGTCCTCCCATGAGGACAAATATCATTTTGGGGAGCAAC 360
2-Sicily                AGCCACAGCATTTATGGGTTATGTCCTCCCATGAGGACAAATATCATTTTGGGGAGCAAC 360
10-Sicily               AGCCACAGCATTTATGGGTTATGTCCTCCCATGAGGACAAATATCATTTTGGGGAGCAAC 360
13-Sicily               AGCCACAGCATTTATGGGTTATGTCCTCCCATGAGGACAAATATCATTTTGGGGAGCAAC 360
8-Sicily                AGCCACAGCATTTATGGGTTATGTCCTCCCATGAGGACAAATATCATTTTGGGGAGCAAC 360
3-Sicily                AGCCACAGCATTTATGGGTTATGTCCTCCCATGAGGACAAATATCATTTTGGGGAGCAAC 360
7-Sicily                AGCCACAGCATTTATGGGTTATGTCCTCCCATGAGGACAAATATCATTTTGGGGAGCAAC 360
4-Sicily                AGCCACAGCATTTATGGGTTATGTCCTCCCATGAGGACAAATATCATTTTGGGGAGCAAC 360
LineB_Oc-cuniculus_     AGCCACAGCATTTATCGGTTATGTCCTCCCATGAGGACAAATATCATTATGGGGAGCAAC 360
CanadaB                 AGCCACAGCATTTATCGGTTATGTCCTCCCATGAGGACAAATATCATTATGGGGAGCAAC 360
South.AfricaB           AGCCACAGCATTTATCGGTTATGTCCTCCCATGAGGACAAATATCATTATGGGGAGCAAC 360
ItalyB                  AGCCACAGCATTTATGGGTTATGTCCTCCCATGAGGACAAATATCATTTTGGGGAGCAAC 352
SwedenB                 AGCCACAGCATTTATGGGTTATGTCCTCCCATGAGGACAAATATCATTTTGGGGAGCAAC 360
1-Sicily                AGCCACAGCATTTATGGGTTATGTCCTCCCATGAGGACAAATATCATTTTGGGGAGCAAC 360
6-Sicily                AGCCACAGCATTTATGGGTTATGTCCTCCCATGAGGACAAATATCATTTTGGGGAGCAAC 360
9-Sicily                AGCCACAGCATTTATGGGTTATGTCCTCCCATGAGGACAAATATCATTTTGGGGAGCAAC 360
5-Sicily                AGCCACAGCATTTATGGGTTATGTCCTCCCATGAGGACAAATATCATTTTGGGGAGCAAC 360
11-Sicily               AGCCACAGCATTTATGGGTTATGTCCTCCCATGAGGACAAATATCATTTTGGGGAGCAAC 360
Lepus.europaeus         GGCTACAGCATTCATAGGCTACGTCCTCCCATGAGGACAAATATCATTCTGAGGGGCTAC 360
Lepus.corsicanus        AGCCACAGCATTTATAGGCTATGTCCTCCCATGAGGACAAATATCATTCTGAGGCGCTAC 355
                         ** ******** *  ** ** ** *********** *********** ** ** ** **

LineA_Oc-algirus_       CGTAATCACTAACCTTCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
MexicoA                 CGTAATCACTAACCTTCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
12-Sicily               CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
2-Sicily                CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
10-Sicily               CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
13-Sicily               CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
8-Sicily                CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
3-Sicily                CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
7-Sicily                CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
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4-Sicily                CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
LineB_Oc-cuniculus_     CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
CanadaB                 CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
South.AfricaB           CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
ItalyB                  CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 412
SwedenB                 CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
1-Sicily                CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
6-Sicily                CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
9-Sicily                CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
5-Sicily                CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
11-Sicily               CGTAATCACTAACCTCCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT 420
Lepus.europaeus         CGTAATTACTAATCTTCTATCAGCCATTCCTTATATCGGAACAACCCTAGTTGAATGAAT 420
Lepus.corsicanus        TGTAATTACTAATCTTTTATCAGCTATCCCCTACATTGGAACAACCCTAGTTGAATGAAT 415
                         ***** ***** **  ******* ** ** ** ** ********* *************

                                      SalI /HincII
LineA_Oc-algirus_       CTGAGGAGGGTTTTCAGTCGACAAAGCCACTCTAACCCGATTTTTCGCTTTTCACTTCAT 480
MexicoA                 CTGAGGAGGGTTTTCAGTCGACAAAGCCACTCTAACCCGATTTTTCGCTTTTCACTTCAT 480
12-Sicily               CTGAGGAGGATTTTCAGTTGATAAAGCTACTCTTACCCGATTCTTCGCTTTTCACTTCAT 480
2-Sicily                CTGAGGAGGATTTTCAGTTGATAAAGCTACTCTTACCCGATTCTTCGCTTTTCACTTCAT 480
10-Sicily               CTGAGGAGGATTTTCAGTTGATAAAGCTACTCTTACCCGATTCTTCGCTTTTCACTTCAT 480
13-Sicily               CTGAGGAGGATTTTCAGTTGATAAAGCTACTCTTACCCGATTCTTCGCTTTTCACTTCAT 480
8-Sicily                CTGAGGAGGATTTTCAGTTGATAAAGCTACTCTTACCCGATTCTTCGCTTTTCACTTCAT 480
3-Sicily                CTGAGGAGGATTTTCAGTTGATAAAGCTACTCTTACCCGATTCTTCGCTTTTCACTTCAT 480
7-Sicily                CTGAGGAGGATTTTCAGTTGATAAAGCTACTCTTACCCGATTCTTCGCTTTTCACTTCAT 480
4-Sicily                CTGAGGAGGATTTTCAGTTGATAAAGCCACTCTTACCCGATTCTTCGCTTTTCACTTCAT 480
LineB_Oc-cuniculus_     CTGAGGAGGATTTTCAGTTGATAAAGCCACTCTTACCCGATTCTTCGCTTTTCACTTCAT 480
CanadaB                 CTGAGGAGGATTTTCAGTTGATAAAGCCACTCTTACCCGATTCTTCGCTTTTCACTTCAT 480
South.AfricaB           CTGAGGAGGATTTTCAGTTGATAAAGCCACTCTTACCCGATTCTTCGCTTTTCACTTCAT 480
ItalyB                  CTGAGGAGGATTTTCAGTTGATAAAGCCACTCTTACCCGATTCTTCGCTTTTCACTTCAT 472
SwedenB                 CTGAGGAGGATTTTCAGTTGATAAAGCCACTCTTACCCGATTCTTCGCTTTTCACTTCAT 480
1-Sicily                CTGAGGAGGATTTTCAGTTGATAAAGCCACTCTTACCCGATTCTTCGCTTTTCACTTCAT 480
6-Sicily                CTGAGGAGGATTTTCAGTTGATAAAGCCACTCTTACCCGATTCTTCGCTTTTCACTTCAT 480
9-Sicily                CTGAGGAGGATTTTCAGTTGATAAAGCCACTCTTACCCGATTCTTCGCTTTTCACTTCAT 480
5-Sicily                CTGAGGAGGATTTTCAGTTGATAAAGCCACTCTTACCCGATTCTTCGCTTTTCACTTCAT 480
11-Sicily               CTGAGGAGGATTTTCAGTTGATAAAGCCACTCTTACCCGATTCTTCGCTTTTCACTTCAT 480
Lepus.europaeus         CTGAGGAGGATTTTCAGTCGATAAAGCCACACTTACCCGATTTTTCGCTTTTCACTTTAT 480
Lepus.corsicanus        TTGAGGAGGGTTCTCAGTCGACAAAGCTACACTCACCCGATTCTTCGCTTTCCACTTCAT 475
                         ******** ** ****** ** ****** ** ** ******** ******** ***** **

                                     AluI
LineA_Oc-algirus_       CCTACCATTTATCATTGCAGCTTTAGTCTTAATTCACCTCCTCTTCCTACATGAAACTGG 540
MexicoA                 CCTACCATTTATCATTGCAGCTTTAGTCTTAATTCACCTCCTCTTCCTACATGAAACTGG 540
12-Sicily               CTTGCCATTTATCATTGCAGCTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 540
2-Sicily                CTTGCCATTTATCATTGCAGCTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 540
10-Sicily               CTTGCCATTTATCATTGCAGCTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 540
13-Sicily               CTTGCCATTTATCATTGCAGCTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 540
8-Sicily                CTTGCCATTTATCATTGCAGCTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 540
3-Sicily                CTTGCCATTTATCATTGCAGCTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 540
7-Sicily                CTTGCCATTTATCATTGCAGCTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 540
4-Sicily                CTTGCCATTTATCATTGCAGCTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 540
LineB_Oc-cuniculus_     CTTGCCATTTATCATTGCAACTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 540
CanadaB                 CTTGCCATTTATCATTGCAACTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 540
South.AfricaB           CTTGCCATTTATCATTGCAACTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 540
ItalyB                  CTTGCCATTTATCATTGCAACTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 532
SwedenB                 CTTGCCATTTATCATTGCAACTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 540
1-Sicily                CTTGCCATTTATCATTGCAACTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 540
6-Sicily                CTTGCCATTTATCATTGCAACTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 540
9-Sicily                CTTGCCATTTATCATTGCAACTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 540
5-Sicily                CTTGCCATTTATCATTGCAACTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 540
11-Sicily               CTTGCCATTTATCATTGCAACTTTAGTCTTAATTCACCTCCTCTTTCTACATGAAACTGG 540
Lepus.europaeus         CCTCCCATTTATTATTGCAGCTCTAGTGATAATTCACTTACTTTTCCTCCATGAAACTGG 540
Lepus.corsicanus        TCTCCCATTCATCATCGCAGCACTAGTGATGATTCACCTACTTTTCCTCCATGAAACTGG 535
                          * ***** ** ** *** *  ****  * ****** * ** ** ** ***********

                                     EcoRI
LineA_Oc-algirus_       TTCCAACAACCCCACAGGAATCCCCTCAAACTCAGATAAAATCCCTTTTCATCCCTACTA 600
MexicoA                 TTCCAACAACCCCACAGGAATCCCCTCAAACTCAGATAAAATCCCTTTTCATCCCTACTA 600
12-Sicily               CTCCAACAACCCCACAGGAATTCCTTCAAACTCAGATAAAATCCCTTTCCACCCCTACTA 600
2-Sicily                CTCCAACAACCCCACAGGAATTCCTTCAAACTCACATAAAATCCCTTTCCACCCCTACTA 600
10-Sicily               CTCCAACAACCCCACAGGAATTCCTTCAAACTCAGATAAAATCCCTTTCCACCCCTACTA 600
13-Sicily               CTCCAACAACCCCACAGGAATTCCTTCAAACTCAGATAAAATCCCTTTCCACCCCTACTA 600
8-Sicily                CTCCAACAACCCCACAGGAATTCCTTCAAACTCAGATAAAATCCCTTTCCACCCCTACTA 600
3-Sicily                CTCCAACAACCCCACAGGAATTCCTTCAAACTCAGATAAAATCCCTTTCCACCCCTACTA 600
7-Sicily                CTCCAACAACCCCACAGGAATTCCTTCAAACTCAGATAAAATCCCTTTCCACCCCTACTA 600
4-Sicily                CTCCAACAACCCCACAGGAATTCCTTCAAACTCAGATAAAATCCCTTTCCACCCCTACTA 600
LineB_Oc-cuniculus_     CTCCAACAACCCAACAGGAATTCCTTCAAACTCAGATAAAATCCCTTTCCACCCCTACTA 600
CanadaB                 CTCCAACAACCCAACAGGAATTCCTTCAAACTCAGATAAAATCCCTTTCCACCCCTACTA 600
South.AfricaB           CTCCAACAACCCAACAGGAATTCCTTCAAACTCAGATAAAATCCCTTTCCACCCCTACTA 600
ItalyB                  CTCCAACAACCCCACAGGAATTCCTTCAAACTCAGATAAAATCCCTTTCCACCCCTACTA 592
SwedenB                 CTCCAACAACCCCACAGGAATTCCTTCAAACTCAGATAAAATCCCTTTCCACCCCTACTA 600
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1-Sicily                CTCCAACAACCCCACAGGAATTCCTTCAAACTCAGATAAAATCCCTTTCCACCCCTACTA 600
6-Sicily                CTCCAACAACCCCACAGGAATTCCTTCAAACTCAGATAAAATCCCTTTCCACCCCTACTA 600
9-Sicily                CTCCAACAACCCCACAGGAATTCCTTCAAACTCAGATAAAATCCCTTTCCACCCCTACTA 600
5-Sicily                CTCCAACAACCCCACAGGAATTCCTTCAAACTCAGATAAAATCCCTTTCCACCCCTACTA 600
11-Sicily               CTCCAACAACCCCACAGGAATTCCTTCAAACTCAGATAAAATCCCTTTCCACCCCTACTA 600
Lepus.europaeus         CTCCAATAACCCATCAGGCATCCCATCAAACTCTGATAAAATTCCATTCCACCCCTACTA 600
Lepus.corsicanus        CTCCAATAATCCATCAGGTATCCCATCAGACTCTGATAAGATTCCATTCCACCCCTATTA 595
                         ***** ** **  **** ** ** *** ****  **** ** ** ** ** ***** **

LineA_Oc-algirus_       TACAATCAAAGACACCTTAGGATTCCTTATAGCCGTTATTCTCCTCCTTATCTTAGTCCT 660
MexicoA                 TACAATCAAAGACACCTTAGGATTCCTTATAGCCGTTATTCTCCTCCTTATCTTAGTCCT 660
12-Sicily               CACAATCAAAGACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 660
2-Sicily                CACAATCAAAGACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 660
10-Sicily               CACAATCAAAGACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 660
13-Sicily               CACAATCAAAGACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 660
8-Sicily                CACAATCAAAGACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 660
3-Sicily                CACAATCAAAGACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 660
7-Sicily                CACAATCAAAGACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 660
4-Sicily                CACAATCAAAGACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 660
LineB_Oc-cuniculus_     CACAATCAAAGACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 660
CanadaB                 CACAATCAAAGACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 660
South.AfricaB           CACAATCAAAGACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 660
ItalyB                  CACAATCAAAGACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 652
SwedenB                 CACAATCAAAGACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 660
1-Sicily                CACAATCAAAGACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 660
6-Sicily                CACAATCAAAGACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 660
9-Sicily                CACAATCAAAGACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 660
5-Sicily                CACAATCAAAGACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 660
11-Sicily               CACAATCAAAAACACCCTAGGTTTCCTTGTAGCCATTCTTCTCCTCCTTATTTTAGTCCT 660
Lepus.europaeus         CACAATTAAAGACGCGCTAGGATTTCTTATACTCATTCTCCTGCTCATACTCCTAGTCTT 660
Lepus.corsicanus        CACAATTAAAGACCTTCTAGGATTTCTCGTACTTATCCTCCTACTCATACTCCTAGTCTT 655
                         ***** *** **    **** ** **  **    *  * ** *** *  *  ***** *

LineA_Oc-algirus_       ATTTTCACCAGATCTACTAGGAGACCCAGACAACTATACCCCTGCTAACCCCCTTAATAC 720
MexicoA                 ATTTTCACCAGATCTACTAGGAGACCCAGACAACTACACCCCTGCTAACCCCCTTAATAC 720
12-Sicily               ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 720
2-Sicily                ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 720
10-Sicily               ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 720
13-Sicily               ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 720
8-Sicily                ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 720
3-Sicily                ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 720
7-Sicily                ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 720
4-Sicily                ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 720
LineB_Oc-cuniculus_     ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 720
CanadaB                 ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 720
South.AfricaB           ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 720
ItalyB                  ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 712
SwedenB                 ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 720
1-Sicily                ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 720
6-Sicily                ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 720
9-Sicily                ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 720
5-Sicily                ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 720
11-Sicily               ATTTTCACCAGACCTATTAGGAGACCCAGACAACTACACCCCTGCCAACCCCCTTAATAC 720
Lepus.europaeus         ATTCTCCCCTGATCTTCTCGGGGACCCAGACAATTATACCCCTGCCAACCCCCTCAACAC 720
Lepus.corsicanus        ATTCTCCCCTGATCTTCTCGGAGACCCAGACAATTACACCCCTGCCAATCCTCTCAACAC 715
                        *** ** ** ** **  * ** ********** ** ******** ** ** ** ** **

LineA_Oc-algirus_       CCCTCCTCATATCAAACCAGAGTGATACTTTCTATTTGCCTATGCTATCCTACGCTCTAT 780
MexicoA                 CCCTCCTCATATCAAACCAGAATGATACTTTCTATTTGCCTATGCTATCCTACGCTCTAT 780
12-Sicily               CCCTCCCCATATCAAACCAGAATGATACTTCCTATTTGCCTACGCTATCCTACGCTCTAT 780
2-Sicily                CCCTCCCCATATCAAACCAGAATGATACTTCCTATTTGCCTACGCTATCCTACGCTCTAT 780
10-Sicily               CCCTCCCCATATCAAACCAGAATGATACTTCCTATTTGCCTACGCTATCCTACGCTCTAT 780
13-Sicily               CCCTCCCCATATCAAACCAGAATGATACTTCCTATTTGCCTACGCTATCCTACGCTCTAT 780
8-Sicily                CCCTCCCCATATCAAACCAGAATGATACTTCCTATTTGCCTACGCTATCCTACGCTCTAT 780
3-Sicily                CCCTCCCCATATCACACCAGAATGATACTTCCTATTTGCCTACGCTATCCTACGCTCTAT 780
7-Sicily                CCCTCCCCATATCAAACCAGAATGATACTTCCTATTTGCCTACGCTATCCTACGCTCTAT 780
4-Sicily                CCCTCCCCATATCAAACCAGAATGATACTTCCTATTTGCCTACGCTATCCTACGCTCTAT 780
LineB_Oc-cuniculus_     CCCTCCCCATATCAAACCAGAATGATACTTTCTATTTGCCTACGCTATCCTACGCTCTAT 780
CanadaB                 CCCTCCCCATATCAAACCAGAATGATACTTTCTATTTGCCTACGCTATCCTACGCTCTAT 780
South.AfricaB           CCCTCCCCATATCAAACCAGAATGATACTTTCTATTTGCCTACGCTATCCTACGCTCTAT 780
ItalyB                  CCCTCCCCATATCAAACCAGAATGATACTTTCTATTTGCCTACGCTATCCTACGCTCTAT 772
SwedenB                 CCCTCCCCATATCAAACCAGAATGATACTTTCTATTTGCCTACGCTATCCTACGCTCTAT 780
1-Sicily                CCCTCCCCATATCAAACCAGAATGATACTTTCTATTTGCCTACGCTATCCTACGCTCTAT 780
6-Sicily                CCCTCCCCATATCAAACCAGAATGATACTTTCTATTTGCCTACGCTATCCTACGCTCTAT 780
9-Sicily                CCCTCCCCATATCAAACCAGAATGATACTTTCTATTTGCCTACGCTATCCTACGCTCTAT 780
5-Sicily                CCCTCCCCATATCAAACCAGAATGATACTTTCTATTTGCCTACGCTATCCTACGCTCTAT 780
11-Sicily               CCCTCCCCATATCAAACCAGAATGATACTTTCTATTTGCCCACGCTATCCTACGCTCTAT 780
Lepus.europaeus         TCCTCCTCACATCAAACCTGAGTGATATTTCCTATTTGCCTACGCCATCCTACGTTCCAT 780
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Lepus.corsicanus          CCCTCCCCATATCAAACCTGAATGGTATTTTCTATTTGCCTACGCCATTTTACGCTCCAT 775
                           ***** ** **** *** ** ** ** ** ********* * ** **  **** ** **
                                                AluI
LineA_Oc-algirus_         TCCAAATAAACTCGGAGGAGTCCTAGCACTAGTTTTATCTATCCTTGTTCTAGCCTTCAT 840
MexicoA                   TCCAAATAAACTCGGAGGAGTCCTAGCACTAGTTTTATCTATCCTTGTTCTAGCCTTCAT 840
12-Sicily                 TCCAAATAAGCTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 840
2-Sicily                  TCCAAATAAGCTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 840
10-Sicily                 TCCAAATAAGCTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 840
13-Sicily                 TCCAAATAAGCTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 840
8-Sicily                  TCCAAATAAGCTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 840
3-Sicily                  TCCAAATAAGCTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 840
7-Sicily                  TCCAAATAAGCTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 840
4-Sicily                  TCCAAATAAGCTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 840
LineB_Oc-cuniculus_       TCCAAATAAACTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 840
CanadaB                   TCCAAATAAACTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 840
South.AfricaB             TCCAAATAAACTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 840
ItalyB                    TCCAAATAAACTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 832
SwedenB                   TCCAAATAAACTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 840
1-Sicily                  TCCAAATAAACTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 840
6-Sicily                  TCCAAATAAACTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 840
9-Sicily                  TCCAAATAAACTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 840
5-Sicily                  TCCAAATAAACTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 840
11-Sicily                 TCCAAATAAACTCGGAGGAGTCCTAGCTCTAGTCCTATCCATCCTTGTTCTAGCCTTCAT 840
Lepus.europaeus           CCCTAATAAACTAGGTGGTGTTCTAGCCCTAGTTATATCAATTCTTATCCTAGCAATTAT 840
Lepus.corsicanus          CCCTAACAAACTAGGAGGCGTTCTAGCCCTAGTTATGTCAATTCTCATCCTAGCAATTAT 835
                           ** ** ** ** ** ** ** ***** *****  * ** ** **  * *****  * **
                                                              DrdI
LineA_Oc-algirus_         CCCATTCCTCCATATATCTAAACAACGTANCATGATATTCCGACCCATTAGCCAAGTCCT 900
MexicoA                   CCCATTCCTTCATATATCTAAACAACGTAGCATGATATTCCGACCCATCAGCCAAGTCCT 900
12-Sicily                 CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 900
2-Sicily                  CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 900
10-Sicily                 CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 900
13-Sicily                 CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 900
8-Sicily                  CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 900
3-Sicily                  CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 900
7-Sicily                  CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 900
4-Sicily                  CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 900
LineB_Oc-cuniculus_       CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 900
CanadaB                   CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 900
South.AfricaB             CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 900
ItalyB                    CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 892
SwedenB                   CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 900
1-Sicily                  CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 900
6-Sicily                  CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 900
9-Sicily                  CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 900
5-Sicily                  CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 900
11-Sicily                 CCCATTCCTTCATATGTCTAAACAACGTAGCATGATATTCCGACCCATTAGTCAAGTCCT 900
Lepus.europaeus           CCCCTTCCTTCATATATCCAAACAACGTAGCATAATATTCCGCCCCATTAGCCAAGTCCT 900
Lepus.corsicanus          CCCCTTCCTCCACATATCCAAACAACGCAGCATAGTATTCCGACCCATTAGCCAAGTCCT 895
                          *** ***** ** ** ** ******** * ***  ******* ***** ** ********
                               EcoRI
LineA_Oc-algirus_         ATTCTGAATCCTCGTCGCAGATCTTCTGACACTGACATGAATCGGGGGTCAGCC 954
MexicoA                   ATTCTGAATCCTCGTCGCAGATCTTCTGACACTGACATGAATCGGGGGTCAGCC 954
12-Sicily                 ATTCTGAGTTCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACC 954
2-Sicily                  ATTCTGAGTTCTCGTCGCAGATCTTCT--------------------------- 927
10-Sicily                 ATTCTGAGTTCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACC 954
13-Sicily                 ATTCTGAGTTCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACC 954
8-Sicily                  ATTCTGAGTTCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACC 954
3-Sicily                  ATTCTGAGTTCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACC 954
7-Sicily                  ATTCTGAGTTCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACC 954
4-Sicily                  ATTCTGAGTTCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACC 954
LineB_Oc-cuniculus_       ATTCTGAGTTCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACC 954
CanadaB                   ATTCTGAGTTCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACC 954
South.AfricaB             ATTCTGAGTTCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACC 954
ItalyB                    ATTCTGAGTTCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACC 946
SwedenB                   ATTCTGAGTTCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACC 954
1-Sicily                  ATTCTGAGTTCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACC 954
6-Sicily                  ATTCTGAGTTCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACC 954
9-Sicily                  ATTCTGAGTTCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACC 954
5-Sicily                  ATTCTGAGTTCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACC 954
11-Sicily                 ATTCTGAGTTCTCGTCGCAGA-------------------------------- 921
Lepus.europaeus           TTTCTGAATCCTTGTCGCAGACCTTCTTACACTCACATGAATTGGAGGACAACC 954
Lepus.corsicanus          CTTCTGAATTCTCGTTGCAGACCTTCTGACACTCACATGGATTGGAGGACAACC 949
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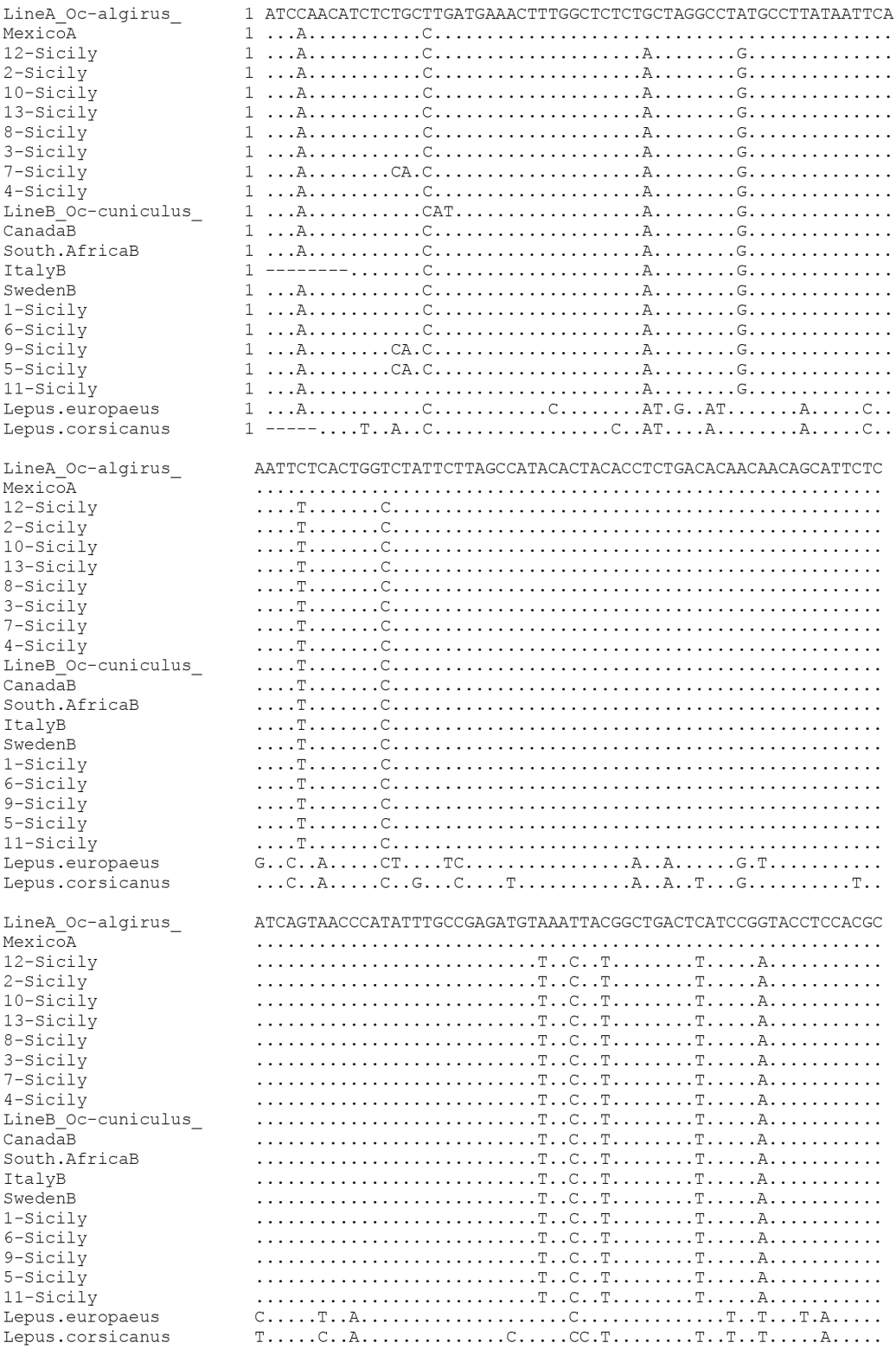

Figure S2. Shading of the multiple alignment of *Oryctolagus cuniculus* cyt *b* mtDNA sequences from different individuals shown in Figure S1. Identical nucleotides are indicated.

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LineA_Oc-algirus_       TAACGGAGCATCTATATTCTTTATTTGTCTTTACATACACGTAGGCCGCGGAATCTACTA
MexicoA                 ..............................................................
12-Sicily               ..........................C..C................................
2-Sicily                ..........................C..C................................
10-Sicily               ..........................C..C................................
13-Sicily               ..........................C..C................................
8-Sicily                ..........................C..C................................
3-Sicily                ..........................C..C................................
7-Sicily                ..........................C..C................................
4-Sicily                ..........................C..C................................
LineB_Oc-cuniculus_     ..........................C..C................................
CanadaB                 ..........................C..C................................
South.AfricaB           ..........................C..C................................
ItalyB                  ..........................C..C................................
SwedenB                 ..........................C..C................................
1-Sicily                ..........................C..C................................
6-Sicily                ..........................C..C................................
9-Sicily                ..........................C..C................................
5-Sicily                ..........................C..C................................
11-Sicily               ..........................C..C................................
Lepus.europaeus         ...T........A..............CT.A..T.....T........T...........
Lepus.corsicanus        C...........A.....T.....C..CT.A..T.....T.....T..T...........

LineA_Oc-algirus_       TGGGTCATACACATACCTAGAAACCTGAAACATCGGCATTATCCTCCTATTCGCAGTGAT
MexicoA                 ..............................................................
12-Sicily               ...A..................G...........T.....C..................A..
2-Sicily                ...A..................G...........T.....C..................A..
10-Sicily               ...A..................G...........T.....C..................A..
13-Sicily               ...A..................G...........T.....C..................A..
8-Sicily                ...A..................G...........T.....C..................A..
3-Sicily                ...A..................G...........T.....C..................A..
7-Sicily                ...A..................G...........T.....C..................A..
4-Sicily                ...A..................G...........T.....C..................A..
LineB_Oc-cuniculus_     ...A..................G...........T.....C..................A..
CanadaB                 ...A..................G...........T.....C..................A..
South.AfricaB           ...A..................G...........T.....C..................A..
ItalyB                  ...A..................G...........T.....C..................A..
SwedenB                 ...A..................G...........T.....C..................A..
1-Sicily                ...A..................G...........T.....C..................A..
6-Sicily                ...A..................G...........T.....C..................A..
9-Sicily                ...A..................G...........T.....C..................A..
5-Sicily                ...A..................G...........T.....C..................A..
11-Sicily               ...A..................G...........T.....C..................A..
Lepus.europaeus         C..C.....T..T.....................T........T..A............A..
Lepus.corsicanus        ...C.....T..T..............G..T..T........T..T.....T.....A..

LineA_Oc-algirus_       AGCCACAGCATTCANGGGATATGTTCTCCCATGAGGTCAAATATCATTTTGAGGAGCAAC
MexicoA                 ..............T...............................................
12-Sicily               ............T.T...T.....C............A..............G........
2-Sicily                ............T.T...T.....C............A..............G........
10-Sicily               ............T.T...T.....C............A..............G........
13-Sicily               ............T.T...T.....C............A..............G........
8-Sicily                ............T.T...T.....C............A..............G........
3-Sicily                ............T.T...T.....C............A..............G........
7-Sicily                ............T.T...T.....C............A..............G........
4-Sicily                ............T.T...T.....C............A..............G........
LineB_Oc-cuniculus_     ............T.TC..T.....C............A...........A..G........
CanadaB                 ............T.TC..T.....C............A...........A..G........
South.AfricaB           ............T.TC..T.....C............A...........A..G........
ItalyB                  ............T.T...T.....C............A..............G........
SwedenB                 ............T.T...T.....C............A..............G........
1-Sicily                ............T.T...T.....C............A..............G........
6-Sicily                ............T.T...T.....C............A..............G........
9-Sicily                ............T.T...T.....C............A..............G........
5-Sicily                ............T.T...T.....C............A..............G........
11-Sicily               ............T.T...T.....C............A..............G........
Lepus.europaeus         G..T..........TA..C..C..C............A...........C.....G..T..
Lepus.corsicanus        ............T.TA..C.....C............A...........C.....C..T..
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LineA_Oc-algirus_       CGTAATCACTAACCTTCTATCAGCAATCCCATATATCGGAACAACCTTAGTTGAATGAAT
MexicoA                 ............................................................
12-Sicily               ..............C.............................................
2-Sicily                ..............C.............................................
10-Sicily               ..............C.............................................
13-Sicily               ..............C.............................................
8-Sicily                ..............C.............................................
3-Sicily                ..............C.............................................
7-Sicily                ..............C.............................................
4-Sicily                ..............C.............................................
LineB_Oc-cuniculus_     ..............C.............................................
CanadaB                 ..............C.............................................
South.AfricaB           ..............C.............................................
ItalyB                  ..............C.............................................
SwedenB                 ..............C.............................................
1-Sicily                ..............C.............................................
6-Sicily                ..............C.............................................
9-Sicily                ..............C.............................................
5-Sicily                ..............C.............................................
11-Sicily               ..............C.............................................
Lepus.europaeus         ......T.....T...........C..T..T.............C.............
Lepus.corsicanus        T.....T.....T...T.......T.....C..C..T.........C.............

LineA_Oc-algirus_       CTGAGGAGGGTTTTCAGTCGACAAAGCCACTCTAACCCGATTTTTCGCTTTTCACTTCAT
MexicoA                 ............................................................
12-Sicily               .........A........T..T.....T.....T........C.................
2-Sicily                .........A........T..T.....T.....T........C.................
10-Sicily               .........A........T..T.....T.....T........C.................
13-Sicily               .........A........T..T.....T.....T........C.................
8-Sicily                .........A........T..T.....T.....T........C.................
3-Sicily                .........A........T..T.....T.....T........C.................
7-Sicily                .........A........T..T.....T.....T........C.................
4-Sicily                .........A........T..T...........T........C.................
LineB_Oc-cuniculus_     .........A........T..T...........T........C.................
CanadaB                 .........A........T..T...........T........C.................
South.AfricaB           .........A........T..T...........T........C.................
ItalyB                  .........A........T..T...........T........C.................
SwedenB                 .........A........T..T...........T........C.................
1-Sicily                .........A........T..T...........T........C.................
6-Sicily                .........A........T..T...........T........C.................
9-Sicily                .........A........T..T...........T........C.................
5-Sicily                .........A........T..T...........T........C.................
11-Sicily               .........A........T..T...........T........C.................
Lepus.europaeus         .........A...........T........A..T.......................T..
Lepus.corsicanus        T...........C..............T..A..C........C........C........

LineA_Oc-algirus_       CCTACCATTTATCATTGCAGCTTTAGTCTTAATTCACCTCCTCTTCCTACATGAAACTGG
MexicoA                 ............................................................
12-Sicily               .T.G.....................................................T.............
2-Sicily                .T.G.....................................................T.............
10-Sicily               .T.G.....................................................T.............
13-Sicily               .T.G.....................................................T.............
8-Sicily                .T.G.....................................................T.............
3-Sicily                .T.G.....................................................T.............
7-Sicily                .T.G.....................................................T.............
4-Sicily                .T.G.....................................................T.............
LineB_Oc-cuniculus_     .T.G.............A.......................................T.............
CanadaB                 .T.G.............A.......................................T.............
South.AfricaB           .T.G.............A.......................................T.............
ItalyB                  .T.G.............A.......................................T.............
SwedenB                 .T.G.............A.......................................T.............
1-Sicily                .T.G.............A.......................................T.............
6-Sicily                .T.G.............A.......................................T.............
9-Sicily                .T.G.............A.......................................T.............
5-Sicily                .T.G.............A.......................................T.............
11-Sicily               .T.G.............A.......................................T.............
Lepus.europaeus         ...C........T.........C....GA........T.A..T.....C...........
Lepus.corsicanus        T..C.....C.....C.....AC....GA.G........A..T.....C...........
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LineA_Oc-algirus_      TTCCAACAACCCCACAGGAATCCCCTCAAACTCAGATAAAATCCCTTTTCATCCCTACTA
MexicoA                ............................................................
12-Sicily              C.....................T..T.......................C..C........
2-Sicily               C.....................T..T.........C............C..C........
10-Sicily              C.....................T..T.......................C..C........
13-Sicily              C.....................T..T.......................C..C........
8-Sicily               C.....................T..T.......................C..C........
3-Sicily               C.....................T..T.......................C..C........
7-Sicily               C.....................T..T.......................C..C........
4-Sicily               C.....................T..T.......................C..C........
LineB_Oc-cuniculus_    C...........A........T..T.......................C..C........
CanadaB                C...........A........T..T.......................C..C........
South.AfricaB          C...........A........T..T.......................C..C........
ItalyB                 C.....................T..T.......................C..C........
SwedenB                C.....................T..T.......................C..C........
1-Sicily               C.....................T..T.......................C..C........
6-Sicily               C.....................T..T.......................C..C........
9-Sicily               C.....................T..T.......................C..C........
5-Sicily               C.....................T..T.......................C..C........
11-Sicily              C.....................T..T.......................C..C........
Lepus.europaeus        C.....T.....AT....C.....A........T........T..A..C..C........
Lepus.corsicanus       C.....T..T..AT....T.....A...G....T.....G..T..A..C..C.....T..

LineA_Oc-algirus_      TACAATCAAAGACACCTTAGGATTCCTTATAGCCGTTATTCTCCTCCTTATCTTAGTCCT
MexicoA                ............................................................
12-Sicily              C..............C....T......G.....A..C.............T........
2-Sicily               C..............C....T......G.....A..C.............T........
10-Sicily              C..............C....T......G.....A..C.............T........
13-Sicily              C..............C....T......G.....A..C.............T........
8-Sicily               C..............C....T......G.....A..C.............T........
3-Sicily               C..............C....T......G.....A..C.............T........
7-Sicily               C..............C....T......G.....A..C.............T........
4-Sicily               C..............C....T......G.....A..C.............T........
LineB_Oc-cuniculus_    C..............C....T......G.....A..C.............T........
CanadaB                C..............C....T......G.....A..C.............T........
South.AfricaB          C..............C....T......G.....A..C.............T........
ItalyB                 C..............C....T......G.....A..C.............T........
SwedenB                C..............C....T......G.....A..C.............T........
1-Sicily               C..............C....T......G.....A..C.............T........
6-Sicily               C..............C....T......G.....A..C.............T........
9-Sicily               C..............C....T......G.....A..C.............T........
5-Sicily               C..............C....T......G.....A..C.............T........
11-Sicily              C.........A.....C....T......G.....A..C.............T........
Lepus.europaeus        C.....T......G.GC.......T......CT.A..C.C..G...A.AC..C.....T.
Lepus.corsicanus       C.....T......CTTC.......T..CG..CTTA.CC.C..A...A.AC..C.....T.


LineA_Oc-algirus_      ATTTTCACCAGATCTACTAGGAGACCCAGACAACTATACCCCTGCTAACCCCCTTAATAC
MexicoA                ...................................C........................
12-Sicily              ...........C...T...................C........C..............
2-Sicily               ...........C...T...................C........C..............
10-Sicily              ...........C...T...................C........C..............
13-Sicily              ...........C...T...................C........C..............
8-Sicily               ...........C...T...................C........C..............
3-Sicily               ...........C...T...................C........C..............
7-Sicily               ...........C...T...................C........C..............
4-Sicily               ...........C...T...................C........C..............
LineB_Oc-cuniculus_    ...........C...T...................C........C..............
CanadaB                ...........C...T...................C........C..............
South.AfricaB          ...........C...T...................C........C..............
ItalyB                 ...........C...T...................C........C..............
SwedenB                ...........C...T...................C........C..............
1-Sicily               ...........C...T...................C........C..............
6-Sicily               ...........C...T...................C........C..............
9-Sicily               ...........C...T...................C........C..............
5-Sicily               ...........C...T...................C........C..............
11-Sicily              ...........C...T...................C........C..............
Lepus.europaeus        ...C..C..T.....T..C..G...........T...........C........C..C..
Lepus.corsicanus       ...C..C..T.....T..C..............T..C........C..T..T..C..C..
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LineA_Oc-algirus_       CCCTCCTCATATCAAACCAGAGTGATACTTTCTATTTGCCTATGCTATCCTACGCTCTAT
MexicoA                 ....................A.....................................
12-Sicily               ......C.............A........C...........C.................
2-Sicily                ......C.............A........C...........C.................
10-Sicily               ......C.............A........C...........C.................
13-Sicily               ......C.............A........C...........C.................
8-Sicily                ......C.............A........C...........C.................
3-Sicily                ......C.......C.....A........C...........C.................
7-Sicily                ......C.............A........C...........C.................
4-Sicily                ......C.............A........C...........C.................
LineB_Oc-cuniculus_     ......C.............A....................C.................
CanadaB                 ......C.............A....................C.................
South.AfricaB           ......C.............A....................C.................
ItalyB                  ......C.............A....................C.................
SwedenB                 ......C.............A....................C.................
1-Sicily                ......C.............A....................C.................
6-Sicily                ......C.............A....................C.................
9-Sicily                ......C.............A....................C.................
5-Sicily                ......C.............A....................C.................
11-Sicily               ......C.............A..................C.C.................
Lepus.europaeus         T........C........T........T..C...........C..C........T..C..
Lepus.corsicanus        ......C...........T..A..G..T..............C..C..TT.......C..


LineA_Oc-algirus_       TCCAAATAAACTCGGAGGAGTCCTAGCACTAGTTTTATCTATCCTTGTTCTAGCCTTCAT
MexicoA                 ............................................................
12-Sicily               .........G.................T.....CC....C...................
2-Sicily                .........G.................T.....CC....C...................
10-Sicily               .........G.................T.....CC....C...................
13-Sicily               .........G.................T.....CC....C...................
8-Sicily                .........G.................T.....CC....C...................
3-Sicily                .........G.................T.....CC....C...................
7-Sicily                .........G.................T.....CC....C...................
4-Sicily                .........G.................T.....CC....C...................
LineB_Oc-cuniculus_     ........................T.....CC....C...................
CanadaB                 ........................T.....CC....C...................
South.AfricaB           ........................T.....CC....C...................
ItalyB                  ........................T.....CC....C...................
SwedenB                 ........................T.....CC....C...................
1-Sicily                ........................T.....CC....C...................
6-Sicily                ........................T.....CC....C...................
9-Sicily                ........................T.....CC....C...................
5-Sicily                ........................T.....CC....C...................
11-Sicily               ........................T.....CC....C...................
Lepus.europaeus         C..T........A..T..T..T.....C......A....A..T...A.C.....AA.T..
Lepus.corsicanus        C..T..C.....A.....C..T.....C......A.G..A..T..CA.C.....AA.T..

LineA_Oc-algirus_       CCCATTCCTCCATATATCTAAACAACGTANCATGATATTCCGACCCATTAGCCAAGTCCT
MexicoA                 .........T.................G.................C..........
12-Sicily               .........T.....G...........G.....................T........
2-Sicily                .........T.....G...........G.....................T........
10-Sicily               .........T.....G...........G.....................T........
13-Sicily               .........T.....G...........G.....................T........
8-Sicily                .........T.....G...........G.....................T........
3-Sicily                .........T.....G...........G.....................T........
7-Sicily                .........T.....G...........G.....................T........
4-Sicily                .........T.....G...........G.....................T........
LineB_Oc-cuniculus_     .........T.....G...........G.....................T........
CanadaB                 .........T.....G...........G.....................T........
South.AfricaB           .........T.....G...........G.....................T........
ItalyB                  .........T.....G...........G.....................T........
SwedenB                 .........T.....G...........G.....................T........
1-Sicily                .........T.....G...........G.....................T........
6-Sicily                .........T.....G...........G.....................T........
9-Sicily                .........T.....G...........G.....................T........
5-Sicily                .........T.....G...........G.....................T........
11-Sicily               .........T.....G...........G.....................T........
Lepus.europaeus         ...C.....T........C..........G...A........C..................
Lepus.corsicanus        ...C........C.....C........C.G...AG.........................
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LineA_Oc-algirus_       ATTCTGAATCCTCGTCGCAGATCTTCTGACACTGACATGAATCGGGGGTCAGCC 954
MexicoA                 ..................................................... 954
12-Sicily               .......G.T.................C.....C...........A..C..A.. 954
2-Sicily                .......G.T.................-------------------------- 927
10-Sicily               .......G.T.................C.....C...........A..C..A.. 954
13-Sicily               .......G.T.................C.....C...........A..C..A.. 954
8-Sicily                .......G.T.................C.....C...........A..C..A.. 954
3-Sicily                .......G.T.................C.....C...........A..C..A.. 954
7-Sicily                .......G.T.................C.....C...........A..C..A.. 954
4-Sicily                .......G.T.................C.....C...........A..C..A.. 954
LineB_Oc-cuniculus_     .......G.T.................C.....C...........A..C..A.. 954
CanadaB                 .......G.T.................C.....C...........A..C..A.. 954
South.AfricaB           .......G.T.................C.....C...........A..C..A.. 954
ItalyB                  .......G.T.................C.....C...........A..C..A.. 946
SwedenB                 .......G.T.................C.....C...........A..C..A.. 954
1-Sicily                .......G.T.................C.....C...........A..C..A.. 954
6-Sicily                .......G.T.................C.....C...........A..C..A.. 954
9-Sicily                .......G.T.................C.....C...........A..C..A.. 954
5-Sicily                .......G.T.................C.....C...........A..C..A.. 954
11-Sicily               .......G.T...........------------------------------- 921
Lepus.europaeus         T...........T........C.....T.....C........T..A..A..A.. 954
Lepus.corsicanus        C........T.....T.....C...........C.....G..T..A..A..A.. 949
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